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Identification by Sequence Analysis of Two-Site Posttranslational Processing of the Cysteine-Rich Outer Membrane Protein 2 of *Chlamydia trachomatis* Serovar L2

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The 60,000-molecular-weight cysteine-rich outer membrane protein (OMP2) from *Chlamydia trachomatis* participates in the disulfide-mediated outer-membrane organization unique to this organism. In addition, this protein is a primary focus of the host immune response. We cloned and sequenced the gene for OMP2 from *C. trachomatis* serovar L2. A λ gt11 recombinant that expressed an antigenic portion of this protein was selected by antibody screening and provided a probe for the selection in λ 1059 of a clone containing the entire gene. DNA sequencing of this clone identified one open reading frame of 1,641 base pairs, starting with a methionine codon and coding for a polypeptide with a molecular weight of 58,792. Amino-terminal protein sequencing and analysis of the translated DNA sequence demonstrated that processing at alternate signal peptide cleavage sites accounts for the molecular-weight polymorphism of this protein. The mature proteins had a net positive charge and contained 24 cysteine residues.

Infections by *Chlamydia trachomatis* are a leading cause of sexually transmitted disease, infertility, and blindness. Within the species, two biovars have been described which are responsible for human disease (19). The trachoma biovar infects mucosal epithelial cells and produces localized infection of the urogenital tract and eyes. The lymphogranuloma venereum (LGV) biovar has broader host cell specificity and causes more invasive sexually transmitted disease. Chlamydiae have a unique life cycle in which the bacteria alternate between two developmental forms, the extracellular elementary body (EB) and the intracellular reticulate body. Only the EB is capable of extracellular survival, host cell recognition, and invasion, suggesting a fundamental role for EB outer membrane constituents in infectivity.

The differences in infectivity observed between the trachoma and LGV biovars may be mediated by dissimilarity in outer membrane composition, variation in primary structure of the EB constituents, or higher-ordered structural heterogeneity. The EB outer membrane contains three cysteine-rich proteins with molecular weights of about 40,000 (MOMP), 60,000 (OMP2), and 15,000. The chlamydial cell envelope lacks a peptidoglycan layer for structural integrity (2). It has been proposed that in its absence inter- and intramolecular disulfide bonding among the cysteine-rich proteins imparts rigidity to the EB outer membrane (22).

Complete sequence information for the MOMP-encoding genes of several serotypes (25, 26) indicates that this protein is antigenically complex, possessing species-, subspecies- and serovar-specific epitopes on each molecule (28). The 15,000-molecular-weight cysteine-rich protein has recently been described as possessing both biovar- and species-specific epitopes (31). To date, the epitopes identified on the 60,000-molecular-weight outer-membrane protein by monoclonal antibodies have all been species specific, demonstrating less antigenic diversity than the other cysteine-rich proteins (20).

Despite the evidence for a shared antigenic structure, there are several striking physical differences between the 60,000-molecular-weight proteins of the trachoma biovar and the more invasive LGV biovar. This protein appears as a predominant single band in gel electrophoresis of trachoma strains but is present as a less intense doublet in LGV strains (3). Recently, peptide maps have demonstrated that there are significant structural differences between the proteins of these two biovars (20). Further, it has been observed that the 60,000-molecular-weight protein of the trachoma biovar has a nearly neutral isoelectric point (pI), whereas the LGV proteins have a net positive charge, with a pI of 8.5 to 9.0 (3). Batteiger et al. (3) have proposed that the relative ease with which LGV strains attach to negatively charged host cells may be aided by this protein. Although a role for this protein in attachment or invasion of host cells has not been elucidated, the physical differences observed between the two biovars in this essential structural protein suggest that it functions as a virulence determinant.

To define the structure, antigenicity, and other potential functions of the 60,000-molecular-weight protein, we isolated and sequenced the gene for this protein from serovar L2 (LGV biovar). In this study, we refer to the 60,000-molecular-weight outer membrane protein as OMP2 because it is the second most abundant outer membrane protein by Coomassie staining. Although, this nomenclature does not include reference to the cysteine-rich nature of this protein, it is appropriate in relation to the MOMP, which is also a relatively cysteine-rich protein (10, 25). The gene for OMP2 from serovar L2 was designated *omp2L2*. *omp2L2* consisted of a 1,641-base-pair (bp) open reading frame which encodes 547 amino acids. The translated amino acid sequence revealed a relatively basic protein containing 24 cysteine residues. Amino-terminal protein sequencing and sequence analysis demonstrated that posttranslational cleavage of a leader peptide occurs at two different sites and results in mature proteins of 54,533 and 56,677 daltons.

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MATERIALS AND METHODS

Bacterial strains. *C. trachomatis* L2/434/Bu, B/TW5/OT, C/TW3/OT, F/UW6/Cx and I/UW-12/Ur have been previously described (15), as has the λ gt11 bacteriophage recombinant from serovar L2 designated gt11/L2/46 (24). Bacteriophage λ 1059, its host *Escherichia coli* Q359 (12), and the pUC plasmids and M13 phage systems, have been previously described (18).

Antiserum production. The β -galactosidase fusion peptide from the induced lysogen of gt11/L2/46 was fractionated by bacterial cell lysis in 1% Zwittergent (Calbiochem-Behring), followed by centrifugation through 20% sucrose at $20,000 \times g$. A rabbit was immunized with about 20 μ g of the fractionated protein subcutaneously with Freund complete adjuvant. The rabbit was boosted intravenously with about 10 μ g of the protein 14 and 21 days after the first immunization. Antiserum was collected 10 days after the last immunization.

Immunoblot analysis. EBs were harvested and Renografin (E. R. Squibb & Sons) purified as previously described (15). Outer membranes were prepared by treatment of EBs with sarcosyl as described by Caldwell et al. (7). Sarcosyl-insoluble pellets were placed in sodium dodecyl sulfate sample buffer containing β -mercaptoethanol, loaded onto polyacrylamide gels, electrophoresed, transferred, and probed by standard methods (24). The rabbit antiserum described above was used as the primary antibody at a dilution of 1/1,000, followed by horse radish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Zymed Labs, Inc.). The chromogen 4-chloro-1-naphthol (Sigma Chemical Co.) was used to detect antibody binding as previously described (24).

Characterization of gt11/L2/46 DNA. DNA was isolated from the recombinant bacteriophage as previously described (16). The insert DNA was identified by digestion of bacteriophage DNA with *Eco*RI and separation on agarose gels. The location of the chlamydial DNA insert relative to *lacZ* was determined by restriction endonuclease analysis with *Sst*I, *Kpn*I, and *Eco*RI. An 870-bp *Eco*RI chlamydial insert from gt11/L2/46 was subcloned into pUC18 and designated J702. Standard methods were used for enzyme analysis and pUC cloning (16).

Construction of genomic libraries for *C. trachomatis* serovar L2. DNA from *C. trachomatis* L2/434/Bu was isolated as previously described (24). Standard procedures were used for enzymatic reactions and for isolation of phage DNA (16). Chlamydial genomic DNA was partially digested with *Sau*3A. The *Sau*3A fragments were ligated to *Bam*HI-digested λ 1059 DNA and packaged into phage by using commercially prepared extracts (Amersham Corp.). Recombinant phage were plated on Q359 for screening (12).

Selection and analysis of λ 1059 recombinant phage. The chlamydial insert DNA from J702 was purified by extraction from low-melting-point agarose and labeled with 32 P. Labeling was performed by the random-primer method with commercially prepared reagents (Bethesda Research Laboratories, Inc.). Phage were plated in *E. coli* Q359 at densities of about 10^3 PFU/150-mm-diameter plate and screened with the 32 P-labeled insert DNA as previously described (25). Plaques that produced strong signals were picked and re-assayed to ensure plaque purity. DNA was isolated from the positive clones as previously described (16). Clones were digested with restriction endonucleases, and restriction fragments of interest were identified by Southern blot analysis (16). *Sst*I fragments of interest were subcloned into pUC18. Two pUC clones, R708 and R709, were mapped by restric-

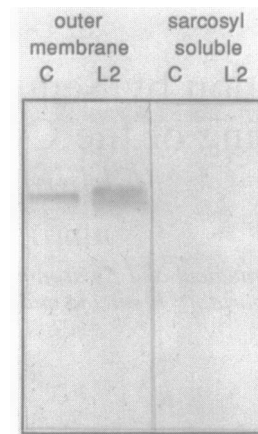


FIG. 1. Immunoblot of *C. trachomatis* antigens; sarcosyl-soluble and sarcosyl-insoluble (outer-membrane) preparations of serovars L2 and C. EB lysates were probed with monospecific rabbit antiserum to the gt11/L2/46 fusion peptide. Following electrophoretic transfer of proteins from a 7.5% polyacrylamide gel to nitrocellulose paper, the sheet was reacted with antiserum, and antibody binding was detected with peroxidase-conjugated anti-rabbit immunoglobulin and 4-chloro-1-naphthol. No reactions were observed in the lanes containing sarcosyl-soluble preparations.

tion endonuclease analysis with *Sst*I, *Pst*I, *Xba*I, *Sal*I, and *Bam*HI.

DNA sequencing. DNA restriction endonuclease fragments to be sequenced were cloned into M13mp18 and M13mp19. The M13 clones were sequenced by the dideoxy-chain termination method by using commercially available reagents (Sequenase; United States Biochemical). When required, oligonucleotide probes were prepared in an automated synthesizer (Biosearch 8600) and used to continue sequencing. In addition, the λ gt11 *Eco*RI site forward primer (New England BioLabs) was used for sequencing of a fragment containing λ gt11 DNA upstream of the *Eco*RI cloning site. All sequences were confirmed by sequencing of the complementary strand.

Protein sequencing. Outer membranes of chlamydial EBs were prepared by treatment with sarcosyl as described by Caldwell et al. (7). Sarcosyl-insoluble pellets were placed in sodium dodecyl sulfate sample buffer containing β -mercaptoethanol, separated on 7.5% polyacrylamide gels, transferred to a membrane (Immobilon; Millipore Corp.), stained with Coomassie brilliant blue, and destained, and the bands were cut out with a razor blade as described by Matsudaira (17). Samples were sent to the Louisiana State University Bio Technologies Unit for sequencing on a pulsed-liquid protein microsequencer (Applied Biosystems model 477A). Amino acid residues were determined from values in the 5- to 10-pmol range.

RESULTS

Characterization of gt11/L2/46. Antiserum to the recombinant bacteriophage gt11/L2/46 (24) was used to probe immunoblots of serovar L2 EB lysates. Two bands with relative mobilities of 56,000 and 58,000 were observed (Fig. 1). Upon fractionation, it was revealed that this antiserum reacted only with the outer membrane (sarcosyl insoluble) of chlamydial EBs. While two bands were observed with serovar L2, the antiserum reacted with only a single 57,000-molecular-weight species in trachoma serovars C and F (Fig. 1; only

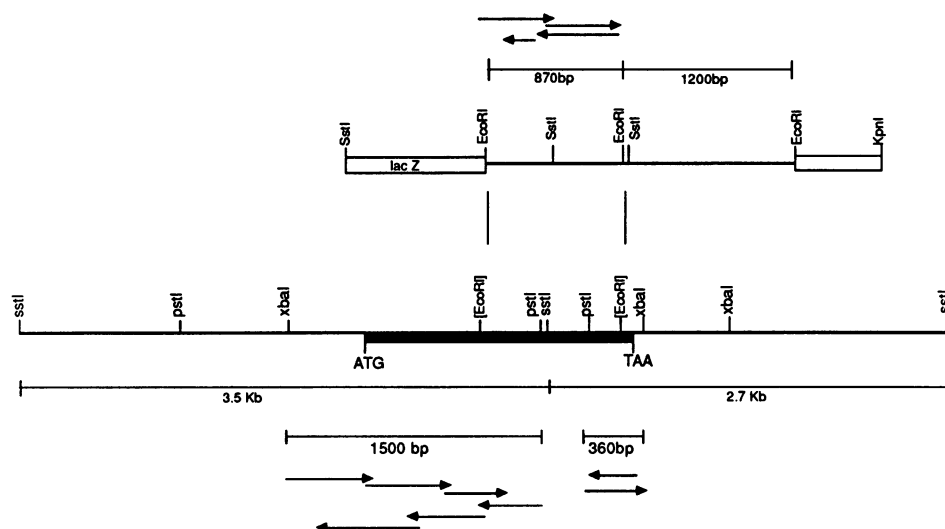


FIG. 2. Restriction endonuclease map of the gt11/L2/46 recombinant clone (top) and the 3.5- and 2.7-kb *SstI* fragments obtained from a λ 1059 recombinant (bottom). The sequencing strategy is shown by arrows. The solid box represents the *omp2* open reading frame. Open boxes represent λ gt11 vector DNA.

serovar C is shown). The fusion peptide from gt11/L2/46 was reactive by immunoblot with one of three monoclonal antibodies that bind distinct epitopes on OMP2 (W. J. Newhall, personal communication). From these data, we concluded that the fusion peptide from this recombinant represents an antigenic portion of the 60,000-molecular-weight outer membrane protein described by several investigators (3, 10, 21).

The fusion peptide had a molecular weight of about 146,000 by polyacrylamide gel electrophoresis, suggesting that the chlamydial DNA insert encodes for a polypeptide with a molecular weight of about 30,000. DNA prepared from gt11/L2/46 was mapped with *KpnI*, *SstI*, and *EcoRI*. An 870-bp *EcoRI* insert adjacent to *lacZ* was identified (Fig. 2).

The 870-bp fragment was cloned into M13mp18 and M13mp19 and sequenced. The sequence revealed only one significant open reading frame, which spanned the entire sequence. In addition, a 1.4-kilobase (kb) *SstI* fragment from gt11/L2/46 (Fig. 2) was cloned into M13mp19. This fragment included a portion of the β -galactosidase gene upstream of the cloning site in addition to the insert DNA. This allowed use of the λ gt11 forward primer, which hybridizes to *lacZ* 13 nucleotides upstream of the *EcoRI* cloning site. A sequence obtained from this clone confirmed that the 870-bp *EcoRI* fragment was adjacent to and in frame with *lacZ*. The molecular weight of the translated open reading frame was 30,416, consistent with that of the protein product. The sequence of this insert DNA is described below with the entire gene sequence.

Selection and analysis of genomic recombinants. The 870-bp chlamydial DNA insert from gt11/L2/46 was used to select recombinants from a λ 1059 genomic library of serovar L2. An *SstI* site had been identified within the gene for OMP2 by mapping of the gt11/L2/46 DNA. Thus, the DNA prepared from these λ 1059 recombinants was digested with *SstI* and evaluated by Southern hybridization. The presence of two bands (2.7 and 3.5 kb) which hybridized to the probe demonstrated that the λ 1059 clones contained the entire gene (data not shown). Both *SstI* fragments were subcloned into pUC18 and mapped (Fig. 2). Restriction sites within the 870-bp fragment and within the two *SstI* fragments allowed orientation of the 3.5-kb fragment 5' to the 2.7-kb fragment.

Restriction endonuclease digests of *C. trachomatis* DNA obtained from serovar L2 were probed by Southern hybridization with the gt11/L2/46 chlamydial DNA insert. The probe hybridized to a single restriction fragment in *XbaI*, *BamHI*, and *HindIII* digests while hybridizing to two bands in *SstI* digests (data not shown), indicating the presence of a single gene for OMP2. Southern hybridization was also performed on *BamHI* and *HindIII* digests of DNA obtained from serovar I. Bands were detected which closely migrated with analogous bands from digests of LGV biovar DNA (Fig. 3). This shows that *omp2* genes for trachoma and LGV biovars are in similar genomic contexts. Thus, gene differences which could account for the physical and structural differences observed in this protein are localized within a common gene framework.

Gene sequence. The sequencing strategy for *omp2*L2 is shown in Fig. 2. The sequence revealed one long open reading frame of 1,641 bp which started with a methionine

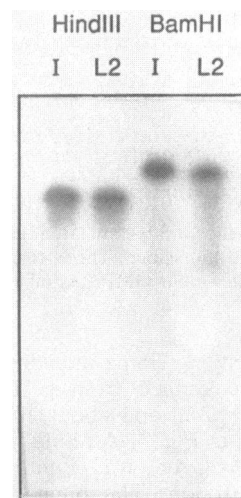


FIG. 3. Southern blot of *HindIII*- and *BamHI*-digested genomic DNAs obtained from *C. trachomatis* serovars L2 and I probed with a 32 P-labeled 870-bp gt11/L2/46 insert.

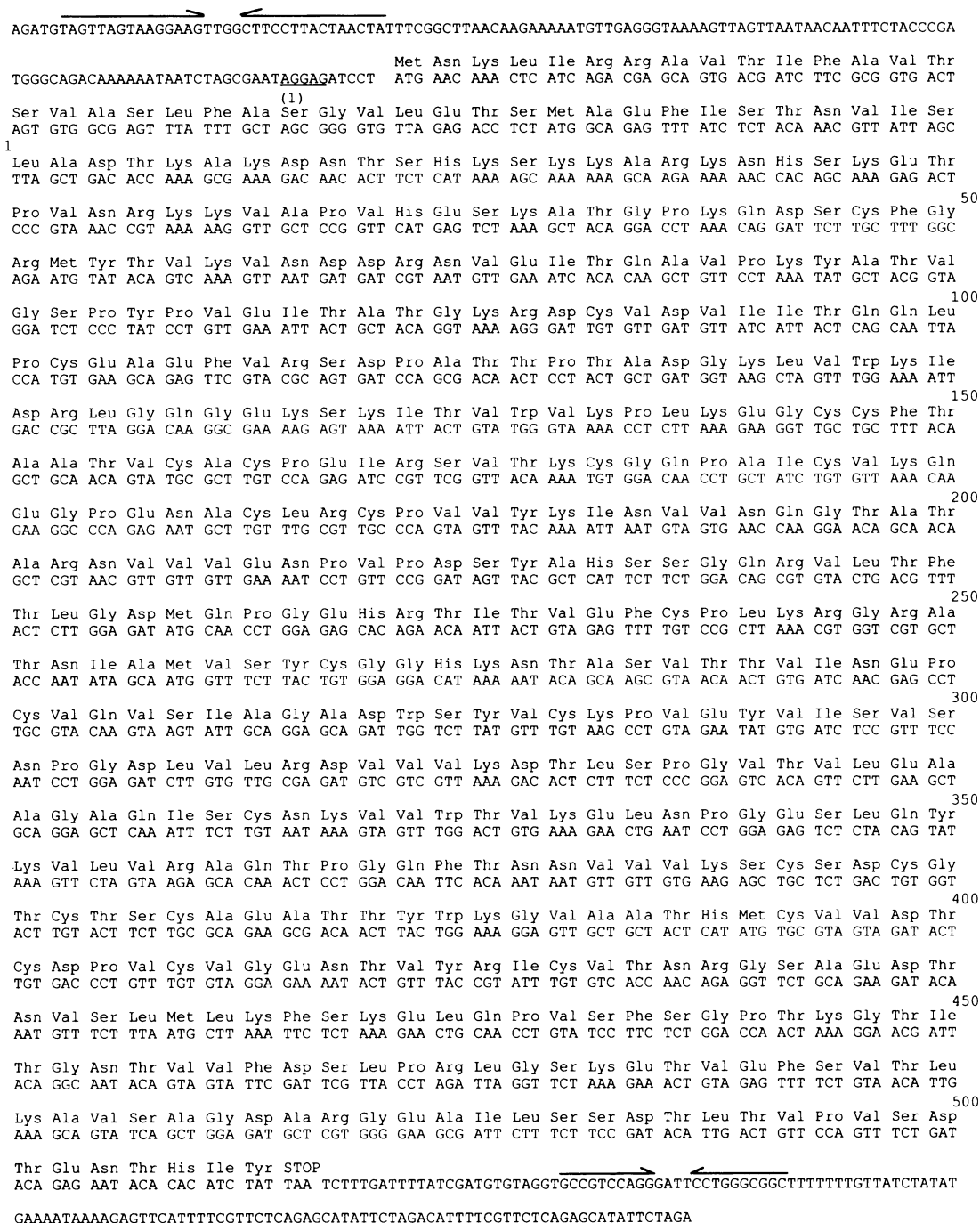


FIG. 4. DNA sequence and translated amino acid sequence of the *omp2L2* gene, including 5' and 3' flanking sequences. The ribosome-binding site is underlined. The N-terminal leucine of the 54,533-molecular-weight OMP_{2_B} is designated 1. The amino-terminal serine of the 56,677-molecular-weight OMP_{2_B} is labeled with the symbol (1). The 5' and 3' dyads are shown by arrows.

codon. This open reading frame included the open reading frame previously sequenced from gt11/L2/46. The open reading frame of *omp2L2* and about 100 bp of a flanking sequence are shown in Fig. 4. A Shine-Dalgarno ribosome-binding sequence, AGGAG, was identified 5 bp upstream from the initiation codon. The open reading frame was terminated by a single stop codon (TAA). Downstream (25 bp) of the stop codon was a G+C-rich 10-bp dyad. This dyad had the potential to form a stem-and-loop structure with a

least-free-energy value of -27.5 kcal (1 cal = 4.184 J) and was followed by seven thymidines, which is compatible with a rho-independent terminator (23). Upstream (97 bp) of the initiation codon was a 16-bp dyad (-28.5 kcal) followed by three thymidines. This is reminiscent of the gene structure of *C. trachomatis omp1* (27). As with the *omp1* sequences (26) and the recently published DNA sequence for a putative *C. trachomatis* binding protein (13), codon usage in this sequence was strongly biased toward U or A in position 3.

Amino acid sequence. The open reading frame of the *omp2L2* gene was translated and, starting with the initial methionine, encoded 547 amino acids (Fig. 4). The molecular weight calculated from the amino acid sequence was 58,792. Analysis of the translated sequence revealed several possible sites for cleavage of a leader peptide for export to the outer membrane. To determine the amino terminus of the mature protein, an outer membrane preparation of serovar L2 was separated on a 7.5% polyacrylamide gel, transferred to an Immobilon membrane, and stained with Coomassie blue, and each band of the OMP2 doublet was excised for microsequencing. The first 11 amino acids of each band in the L2 doublet were determined. The sequence derived from the lower-molecular-weight band (Leu-Ala-[Asp or Asn]-Thr-Lys-Ala-[Lys or Leu]-X-Asn-Thr-Ser) corresponded to amino acids 41 through 51 following the initial methionine in the translated sequence. A sequence derived from the higher-molecular-weight band (X-Gly-Val-Leu-Glu-Thr-X-Met-Ala-Glu) corresponded to amino acids 23 to 33 of the translated open reading frame. The results revealed that the molecular-weight polymorphism observed in LGV OMP2 was due to differences in posttranslational cleavage of the precursor protein, yielding two different amino termini. The 547-amino-acid protein encoded by the gene can be processed at two signal sequence cleavage sites to yield either a 507-amino acid or a 525-amino-acid mature protein. Protein gels indicate that this occurs in nearly equimolar ratios. Molecular weights derived from translation of the *omp2* open reading frame, starting at the signal cleavage sites, are 54,533 (OMP2_α) and 56,677 (OMP2_β). The relative mobilities for OMP2 observed by polyacrylamide gel electrophoresis, 59,000 and 62,000 (10), may be artifactually high, since overestimation of mass is a common feature of gel electrophoresis systems. The first 17 amino-terminal residues of the trachoma serovar F mature OMP2 were determined by W. J. Newhall (personal communication) and are identical to the amino-terminal residues of serovar L2 OMP2_α. Although a 40-amino-acid leader peptide is exceptionally long, both OMP2L2 signal sequence cleavage sites follow the -1, -3 rule described by von Heijne (29, 30). In addition, the cleavage site resulting in OMP2_β is the same at positions -1 and -3 (Leu-X-Ala) as that of the chlamydial MOMP leader peptide (25), suggesting that OMP2 biovar differences are not due to the inability of trachoma strains to cleave at that site.

The amino acid composition of serovar L2 OMP2, as determined by translation of the open reading frame (Table 1), included an excess of basic residues, consistent with the basic charge observed by isoelectric focusing techniques (3). In addition, the two glutamic acid residues present in the 18 additional amino acids of OMP2_β account for the slight isoelectric point difference observed between the OMP2 doublet bands. The translated open reading frame consisted of 24 cysteines (about 5%), which confirmed the cysteine-rich nature of this protein as described by Hatch et al. (10). Both the amino-terminal and carboxyl-terminal ends (within about 90 amino acids) were notably lacking in cysteine residues, and cysteine residues tend to be clustered in two regions within the protein (Fig. 5). Of 24 cysteine residues, 15 were within four amino acid residues of a proline, and computer-assisted secondary structure evaluation (1) revealed that nearly every cysteine residue was immediately preceded by or fell within a beta turn (data not shown). This suggests that cysteine residues are near flexible peptide boundaries which may be important for disulfide-mediated membrane protein interactions.

TABLE 1. Amino acid composition of serovar L2 OMP2

| Amino acid (% of total residues) | No. of residues ^a |
|-------------------------------------|---------------------------------|
| Basic (12.4) | |
| Lys..... | 38 (1) |
| His..... | 8 |
| Arg..... | 22 |
| Acidic (9.7) | |
| Asp..... | 24 |
| Glu..... | 29 |
| Polar (38.0) | |
| Asn..... | 25 (2) |
| Cys..... | 24 |
| Gln..... | 16 |
| Gly..... | 34 (1) |
| Ser..... | 42 (6) |
| Thr..... | 55 (4) |
| Tyr..... | 12 |
| Hydrophobic (39.9) | |
| Ala..... | 42 (5) |
| Ile..... | 23 (4) |
| Leu..... | 27 (3) |
| Met..... | 7 (2) |
| Phe..... | 13 (3) |
| Pro..... | 29 |
| Trp..... | 5 |
| Val..... | 72 (5) |

^a Numbers in parentheses indicate the amino acid composition of the 40-amino-acid leader peptide.

In general, OMP2 has a very even distribution of charged, polar, and hydrophobic residues, with two exceptions. The most notable is the positively charged, highly polar amino terminus (residues +3 to +25) (Fig. 5). This 23-amino-acid stretch includes 11 charged residues (mostly lysines) and is uninterrupted by truly hydrophobic amino acids. There is one polar but not highly charged region of 18 amino acids (+369 to +411) within the polypeptide which overlaps the second cluster of cysteines. No significant stretches of hydrophobic amino acids were evident.

DISCUSSION

OMP2 of *C. trachomatis* functions as a key structural component of the unique chlamydial outer membrane which, in the absence of peptidoglycan, maintains rigidity via disulfide bonding of outer membrane proteins. Further, the cysteine-rich nature of this protein and its expression late in the developmental cycle suggest involvement in the disul-

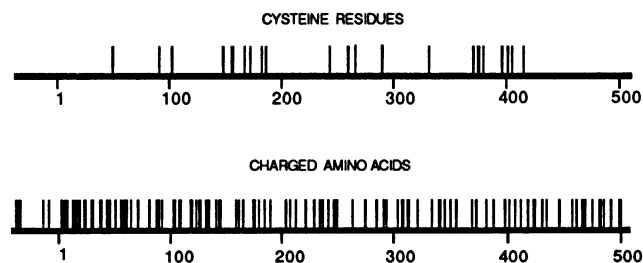


FIG. 5. Linear distribution of cysteine residues and charged residues for the L2 OMP2 determined by translation of the *omp2L2* gene. Each line represents one amino acid.

fide-mediated changes that are essential for the switch between intracellular and extracellular stages of this organism (4, 9). The antigenic significance of this protein in the host immune response is demonstrated by studies in which the presence of antibodies to OMP2 is consistently observed in active infection (5, 8, 21) and is correlated with disease status (5, 6, 11). Although no differences in antigenicity have been observed between the OMP2s of trachoma and LGV biovars (3, 20), significant differences in physical (20) and chemical (3) properties of this protein between biovars have been reported. The OMP2s of LGV and trachoma share important and conserved structural functions; thus, dissimilar properties could be related to the differences in infectivity observed between the two biovars.

EBs of the LGV biovar readily infect cell monolayers without the assistance of centrifugation or host cell pretreatment with polycations required by the trachoma biovar EBs. The OMP2 doublet of LGV biovar strains has been shown by Batteiger et al. (3) to have a pI of 8.5 to 9.0 as opposed to the nearly neutral pI of the trachoma biovar OMP2. They proposed that OMP2 may aid EB attachment to host cells by providing a local region of positive charge and that the difference in infectivity between biovars is due to the greater positive charge of the LGV OMP2 than that of the trachoma OMP2. Since the *omp2* genes are highly conserved in genetic context between the biovars and the proteins are antigenically very similar, the biovar differences observed in OMP2s are probably localized to a defined domain(s) within the proteins. Protein sequencing of the serovar F (trachoma biovar) amino terminus (Newhall, personal communication) demonstrates that these differences are not localized to this charged region. Immunoblots with OMP2-specific antiserum show that trachoma OMP2 migrates midway in molecular weight between LGV OMP2_α and OMP2_β, and yet the amino terminus of trachoma OMP2 is the same as that of LGV OMP2_α. This suggests that trachoma OMP2 possesses a highly acidic region(s), absent in LGV strains, which accounts for both the molecular-weight and charge differences observed between these otherwise identical proteins. Trachoma gene differences may also account for the slight size difference observed in the *Bam*HI restriction fragments by Southern analysis (Fig. 3).

One of the distinctive features of the OMP2 of the LGV biovar strains is the presence of two antigenically identical bands which differ in molecular weight (3). Southern analysis demonstrated that the two bands seen on protein gels were not derived from two genes. Protein sequencing of the two bands and analysis of the translated open reading frame revealed that the protein is cleaved at two alternate peptide cleavage sites, removing either a 22-amino-acid or a 40-amino-acid leader sequence. However, the existence of OMP2_β in the outer membrane suggests that 22 amino acids are sufficient for translocation to the outer membrane. Therefore, cleavage may be sequential with the removal of a 22-amino-acid leader peptide from all precursors, with half of the proteins undergoing further cleavage of 18 amino acids. Removal of the additional 18 amino acids would probably play a subsequent role, perhaps in assembly of these proteins in the outer membrane.

Two-site posttranslational cleavage of a single integral membrane protein precursor has not been described in other procaryotes; thus, this represents a novel mechanism with potentially important microbiological roles. This mechanism, in which structural heterogeneity is derived from the product of a single gene, may allow LGV OMP2s to exist in two conformations or to exist in two different orientations in

the outer membrane. This could modify the higher-ordered disulfide interactions in the outer membrane of the LGV biovar and affect surface exposure of functionally important domains. For example, following translocation, the LGV proteins may form dimers in which one of the two amino-terminal ends is not accessible for second-site cleavage. This would explain the presence of equimolar quantities of OMP2_α and OMP2_β in the outer membrane. Sequential cleavage may also occur in trachoma strains, but because of fundamental differences in structure between the LGV and trachoma OMP2s, trachoma biovar OMP2s are all accessible for second-site cleavage. Analogous OMP2_α and OMP2_β proteins are seen in *C. psittaci* (10). Conservation of OMP2 polymorphism between species which exhibit similar culture infectivity characteristics but are otherwise highly divergent (19) suggests that this mechanism may be important in chlamydial virulence.

Delineation of the primary sequences of the serovar L2 OMP2 and the *omp2L2* gene, as well as the availability of cloned reagents, provides the basis for addressing questions concerning the function and regulation of this protein. Expression of this cloned gene in *E. coli*, alone or in combination with other cloned genes, may allow a more direct evaluation of potential function in host cell attachment, endocytosis, or inhibition of lysosomal fusion. In combination with the sequences for MOMP and the 15,000-molecular-weight cysteine-rich protein, it may be possible to derive structural models for the disulfide interactions in the outer membrane. Partial sequence data reveal a cysteine-rich open reading frame upstream of the *omp2L2* gene, and Northern (RNA) hybridization data indicate that the transcript for the *omp2L2* gene is sufficiently large to encode an additional protein (data not shown). These preliminary findings suggest the possibility of an operon that encodes two EB-specific, cysteine-rich outer-membrane proteins.

Most studies of antigen-specific antibody responses during chlamydial infection have noted OMP2 as a major immunoreactive component (5, 6, 8, 21). These observations have led to interest in the potential role of this antigen in both immunologically mediated protection and pathogenicity. A strong correlation between the immunogenicity of a peptide and the tendency or ability to form a β turn has been described (14). The frequency of β turns in OMP2 as predicted by computer analysis may in part explain the marked immunogenicity of this protein. The use of sequence-defined antigens derived from the cloned *omp2L2* gene can be used to investigate these mechanisms at both the B- and T-cell levels. In addition, Newhall et al. (21) have proposed that this antigen may be a useful serodiagnostic marker of infection. Cloned OMP2 antigens can be used for this purpose without the potential problem of Fc binding described for OMP1 (21).

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